

USE OF INNOVATIVE CULTURES OF MICROORGANISMS IN THE TECHNOLOGY OF FERMENTED BEVERAGES

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Abstract. *The article presents the results of theoretical and experimental research on the expediency of using dry leavens and yeast cultures in bread kvass technology, which are used in various branches of the food industry and at the household level. The characteristics of yeast and lactic acid bacteria used in the technologies of fermented beverages are given. The possibility of using dry sourdoughs Acidolact VIVO, Yogurt VIVO, Kvass VIVO, Bifivit VIVO, Streptosan VIVO to ensure the intensification of the production process of bread kvass has been determined. The characteristics of sourdough starters, the content of microorganism cultures in them, the prospects of using them for fermenting sourdough wort are given.*

Comparative characteristics of the use of pure yeast culture Saccharomyces cerevisiae MP-10 with dry starters are given. This can be important in industrial technology. The dynamics of changes in dry matter and titrated acidity during the fermentation of fermented wort with certain starter cultures were studied. Fermentation took place using a pure yeast culture Saccharomyces cerevisiae MP-10 at temperatures of 30 and 36 °C. The influence of temperature on the physiological activity of yeast during combined alcoholic and lactic acid fermentation under different technological regimes was investigated. The organoleptic parameters of bread kvass using sourdough starters were determined. Based on the results of research into the wort fermentation process and organoleptic evaluation of the finished product, recommendations for the industrial production of bread kvass were provided. Manufacturers of kvass bread using dry starters are recommended to use a pure culture of yeast Saccharomyces cerevisiae MP-10. It has been proven that the use of this yeast culture in combination with dry starters allows to intensify the technological process, in particular at elevated temperatures, to improve the organoleptic qualities of finished products.

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Introduction. An important direction of the development of the beverage industry in the world is the increase in the production of food products with a low sugar content and intended for the prevention of various diseases and used in environmentally unfavorable conditions (Giri et al., 2023). Numerous studies in the field of nutrition physiology show that the most rational form of such products are soft drinks (Basinskiene et al., 2020, Jordana, 2000).

According to the current standard, soft drinks include: juice drinks, drinks based on spicy and aromatic raw materials, drinks based on flavors, drinks based on grain raw materials, drinks based on mineral waters, special purpose drinks, artificially mineralized waters and fermented drinks (fermented drinks). There is a steady trend in the world to increase the production of soft drinks and expand their assortment. However, their biological value, balanced composition, as well as adaptability to the needs of the human body need to be improved. These issues have been resolved in

the countries of Western Europe and Japan, where the composition of drinks has been brought into line with scientifically based norms of consumption of biologically active substances (Dulka et al., 2019).

The range of non-alcoholic beverages in the developed countries of the world is quite wide: from drinks like cola to natural fruit and vegetable juices. It should be noted that their basis is mainly concentrated juices, essences, mineral waters, and artificial compositions. Fermented soft drinks are insufficiently produced. This indicates a certain one-sidedness of their production in the world. The situation is somewhat better in the countries of Eastern Europe due to the production of beverages based on bread raw materials. In particular, bread kvass, a traditional drink for this region.

Non-alcoholic fermented drinks are a food product of plant origin. Their organoleptic and physicochemical properties are formed as a result of the vital activity of cultures of microorganisms substances (Branyik et al., 2012).

Until the beginning of the 20th century, fermented rye kvass was used as a fermentation agent because it contained microorganisms. Such leavens were a mixture of different types of yeast and acid-forming bacteria. When introduced into the nutrient medium, they caused its fermentation. But this method is unacceptable in industrial use, because from the point of view of technological and microbiological aspects, consistently high organoleptic and regulatory physico-chemical indicators of the finished product are not provided. Therefore, in the production of fermented beverages, it is desirable to use only pure cultures of microorganisms, regardless of whether it is a monoculture or an association of cultures of microorganisms (Sõukand et al., 2015, Marsh et al., 2014).

For the fermentation of kvass wort, different breeds of kvass yeast are used. You can also use pressed or dry baker's yeast. The use of pure cultures of yeast races R-87, K-87, KM-94 is considered the most effective in the production of bread kvass. They allow to simplify the technology, to achieve high indicators of kvass (Dulka, 2019).

Yeasts are simpler unicellular organisms belonging to the class *Saccharomyces*. They cause alcoholic fermentation of wort carbohydrates, which occurs under the influence of yeast cell enzymes. The role of yeast in kvass technology is decisive, since the quality of the finished product depends on it. Mainly its taste-aromatic properties. When choosing a yeast strain for the fermentation of fermented wort, it is necessary to take into account their technological properties: high fermentation activity, resistance to autolysis, the ability to form a dense sediment after fermentation, the ability to give the product excellent taste and aroma qualities (Vitriak, 2002).

The most common is the use of dry or pressed baker's yeast. Their use ensures acceptable fermentation of wort, but at the same time the organoleptic indicators of kvass and its stability are low (Dulka, 2019, Semenov et al., 2019).

When using wine yeast, the rate of fermentation slows down significantly, as their enzymatic system is adapted to the fermentation of fruit must.

Breeds of brewer's yeast have acceptable fermentation activity and are closest in characteristics to kvass yeast, but are adapted to a higher dry matter content in the original wort (Rana et al., 2020).

Kvass yeasts are facultative anaerobes and ferment glucose, sucrose, maltose and raffinose to a lesser extent, and partially ferment dextrins, which allowed them to be classified as representatives of *Saccharomyces cerevisiae*, not *Saccharomyces minor*, as previously believed. They do not assimilate lactose, arabinose, xylene, mannitol. Their cells, after cultivation on kvass wort for 24 hours, have dimensions of $6.3 \dots 7.5 \times 5 \dots 7 \text{ }\mu\text{m}$. The temperature optimum for yeast development is

25...30 °C (Dulka, 2019, García et al., 2019).

When preparing kvass using only yeast cultures, there is no accumulation of lactic acid. This happens due to the absence of lactic acid fermentation. Therefore, the necessary conditions of kvass are achieved by blending the fermented wort with organic acids (Dulka, 2019). The use of cultures of lactic acid bacteria in the process of wort fermentation is more appropriate in comparison with artificial acidification. In addition, such combined fermentation prevents the formation of extraneous microflora in the fermentation process and significantly reduces the risk of infection of the finished product (Taco et al., 2021).

Lactic acid bacteria mainly belong to the genus *Streptococcus* and are spherical or oval in shape. Or to the genus *Lactobacillus*, which are immobile non-spore-forming short rods. All of them are gram-positive microorganisms (Hati et al., 2019).

The most famous lactic acid bacteria used in bread kvass technology are β -bacteria of races 11 and 13, which belong to the genus *Lactobacillus*. In the wort, these bacteria look like rods connected in pairs or in short chains. After cultivation for 24 hours, the cells of these bacteria have a length of 1.2...2.0 μm and a width of 0.5...0.6 μm . They are anaerobes, mesophiles, and belong to bacteria of the heteroenzymatic type of carbohydrate decomposition. During fermentation, acetic acid and carbon dioxide are first formed, and lactic acid accumulates at the end. As a result of fermentation of sour wort, bacteria produce lactic, oxalic, acetic, malic and other acids, as well as ethyl alcohol and carbon dioxide. These microorganisms ferment maltose, maltotriose, and sucrose well. The temperature optimum for vital activity is 30...35 °C (García et al., 2019).

The most promising technologies of fermented beverages are those whose technology involves the use of several cultures of microorganisms belonging to different taxonomic groups, including yeast and lactic acid bacteria.

Fermentation of kvass wort has its own characteristics. This is due to the biochemical composition of the raw materials, the features of the joint development of yeast and lactic acid bacteria, and the incompleteness of the process. As a result of fermentation, fermented wort is biotransformed into a finished drink with original taste and aroma properties (Vitriak, 2002).

Lactic acid bacteria differ from yeast in their high demands on the composition of the nutrient medium. They need a complete composition of amino acids and vitamins of group B. Therefore, protein hydrolysates or yeast extracts, vitamins are necessary for their development. That is, substances formed in the process of yeast autolysis. However, the metabolism of lactic acid bacteria leads to an increase in the acidity of the environment, which negatively affects the vital activity of yeast cells and can cause a slowdown in their fermentation activity.

The joint development of yeast and lactic acid bacteria is based on the mutual exchange of nutrients, different requirements for the composition of the wort and the speed of reproduction. As a result of joint cultivation, the direction of their characteristic fermentation changes. In the first half of the combined fermentation process, as a result of the life activity of lactic acid bacteria, lactic acid accumulates and the acidity of the environment increases (up to pH 5.0...5.5), favorable conditions for the life activity of yeast are created. In the second half of the fermentation process, the further increase in acidity inhibits the vital activity of yeast cells and they begin to die. The products of autolysis of yeast become a nutrient medium for lactic acid bacteria, which, when the process is carried out for a long time, leads to the termination of the vital activity of yeast (Dulka et al., 2019).

In order to balance the development of yeast and lactic acid bacteria at the wort fermentation stage, production cultures must be prepared separately and under optimal conditions. At the same

time, it is necessary to control the acidity of lactic acid wort and the concentration of yeast cells in the wort. Therefore, introduction of production cultures of yeast and lactic acid bacteria should be carried out separately, depending on their physiological state (Jordana, 2000).

For enterprises of small and medium capacity (up to 1,000 dal per day), the use of a clean culture department in the production structure is impractical, as it requires significant material and labor resources. Therefore, such enterprises use dry or pressed baker's yeast for the fermentation of kvass wort. To give the drink the necessary acidity, acidification of the finished product is used by adding lactic or citric acid (Gran et al., 2003).

Currently, the consumer market of various leavens for the preparation of fermented drinks at home is represented quite widely. The sourdough market includes sourdoughs for obtaining fermented milk products, including yogurts, sour cream, kefir, and various types of cheeses. The composition of these leavens is diverse and is represented by yeast and bacteria. These microorganisms have different physiology and form different qualitative composition of the finished product and its organoleptic and physicochemical indicators.

Thus, the research and selection of dry cultures of yeast and lactic acid bacteria for the production of bread kvass is relevant.

The purpose of the work is the research and selection of dry leavens, which are used for the preparation of fermented milk products and have a wide range of cultures of microorganisms, for the preparation of bread kvass. Sourdough research will be conducted in combination with *Saccharomyces cerevisiae* MP-10 yeast culture, which is a traditional yeast race for kvass preparation (Dulka, 2019).

Materials and methods. The researches used: dry commercial leavens and pure MP-10 yeast culture according to passport data, drinking water from the centralized water supply of the city of Kyiv according to DSanPiN 2.2.4-171-10, white sugar according to DSTU 4623-2006, kvass wort concentrate in accordance with current regulatory documentation.

During the research, methods generally accepted in the beer-non-alcoholic industry of the food industry were used.

Samples were prepared for research using starter cultures:

- 1 - VIVO Acidolact - "Narine" sourdough starter;
- 2 - VIVO Yogurt - sourdough starter for yogurt;
- 3 – Kvass VIVO – starter for kvass (control);
- 4 - Bifivit VIVO - sourdough starter for children's sour milk nutrition;
- 5 – Streptosan VIVO – sourdough starter for Streptosan sour milk drink.

Working suspensions of *Saccharomyces cerevisiae* MP-10 yeast at the rate of 8% of the wort volume were added to samples 1, 2, 4, 5. Sample 3 was used as a control, yeast was not added.

The initial indicators of fermented wort for all samples were: dry matter content – 3.3%; acidity - 1.15 cm³ of NaOH solution conc. 10 mol/dm³ per 100 cm³.

The wort was fermented at temperatures of 30 and 36 °C for 24 hours. Fermentation was terminated when the dry matter content of the wort decreased by 0.8...1.0% and the acidity increased by 2.0...2.5 cm³ of NaOH solution with a concentration of 1.0 mol/dm³ per 100 cm³.

The physiological state of yeast cultures and their concentration were determined by microscopy using a Horyaev camera. A solution of methylene blue was used to determine the number of dead cells.

Experiments were performed in identical conditions, in three to five repetitions. The given

research results are the mean value of the obtained results.

Results and discussion. The choice of dry sourdoughs was due to their wide use in various branches of the food industry and in everyday life, taking into account the fact that they were not used in the production of bread kvass.

The characteristics of the studied preparations of dry starters by species composition are given in Table 1 (VIVO, 2023).

Table 1

Characteristics of dry sourdough starters

Sam ple №	Name sourdough	Specific composition of the sourdough	Characteristics of sourdough
1.	Acidolact VIVO	<i>Lactobacillus acidophilus</i> <i>Streptococcus salivarius subsp.</i> <i>termophilus</i> ; <i>Lactococcus lactis subsp.</i> <i>diacetulactis</i> .	It is recommended for use after antibiotics or chemotherapeutic drugs, as a support for the body's microflora. The bacteria that make up the sourdough are resistant to most types of antibiotics and are able to inhibit the development of pathogenic microorganisms. It has a complex anti-inflammatory effect, neutralizes toxins and side effects of food products, activates the body's cleansing processes.
2.	Yogurt VIVO	<i>Streptococcus salivarius subsp.</i> <i>termophilus</i> ; <i>Lactobacillus delbrueckii</i> <i>subsp. bulgaricus</i> ; <i>Lactobacillus acidophilus</i> ; <i>Lactococcus lactis subsp.</i> <i>lactis</i> ; <i>Lactococcus lactis subsp.</i> <i>diacetylactis</i> ; <i>Lactococcus lactis subsp.</i> <i>cremoris</i> .	Recommended for people of all ages. Consumption of the product satisfies the body's need for amino acids, calcium salts, vitamins, etc. Useful for people with increased physical or psychological stress. Normalizes digestion, promotes the removal of harmful substances from the body, strengthens immunity. Contains a significant amount of lactic acid, which suppresses the development of pathogenic bacteria in the body
3.	Kvass VIVO	<i>Lactobacillus acidophilus</i> ; <i>Streptococcus salivarius subsp.</i> <i>termophilus</i> ; microflora of kefir fungi; dried baker's yeast.	The product normalizes digestion, promotes the removal of harmful substances from the body, has the ability to heal wounds, restore the microbiocenosis in the intestine, fight gastritis and colitis, and promote the rejuvenation of the body.
4.	Bifivit VIVO	<i>Acetobacter aceti</i> ; <i>Bifidobacterium bifidum</i> ; <i>Bifidobacterium longum</i> ; <i>Bifidobacterium adolescentis</i> ; <i>Lactococcus lactis ssp.</i>	The product is highly effective in the prevention and treatment of diseases of the gastrointestinal tract, effective in staphylococcal infection, allergies, respiratory diseases, impaired immunity and

		<i>cremoris</i> ; <i>Lactococcus lactis ssp. diacetylactis</i> ; <i>Propionibacterium freudenreichii</i> .	metabolism. Restores healthy intestinal microflora in case of dysbacteriosis.
5.	Streptosan VIVO	<i>Streptococcus salivarius subsp. termophilus</i> ; <i>Enterococcus faecium</i> .	Sourdough cultures are part of the microflora of Caucasian fermented milk products such as matsoni and suluguni. The product has the ability to resist the pathogens of intestinal infections and putrefactive bacteria, normalizes metabolism, the work of the cardiovascular system, and prevents premature aging of the body.

Indicators of dry starters Acidolact VIVO, Yogurt VIVO - sourdough starter for yogurt, Kvass VIVO, Bifivit VIVO, Streptosan VIVO and the possibility of their use in the technology of bread kvass are given.

The dynamics of changes in the concentration of dry substances of kvass wort during fermentation with the tested starter samples at a fermentation temperature of 30 °C are shown in Figure 1.

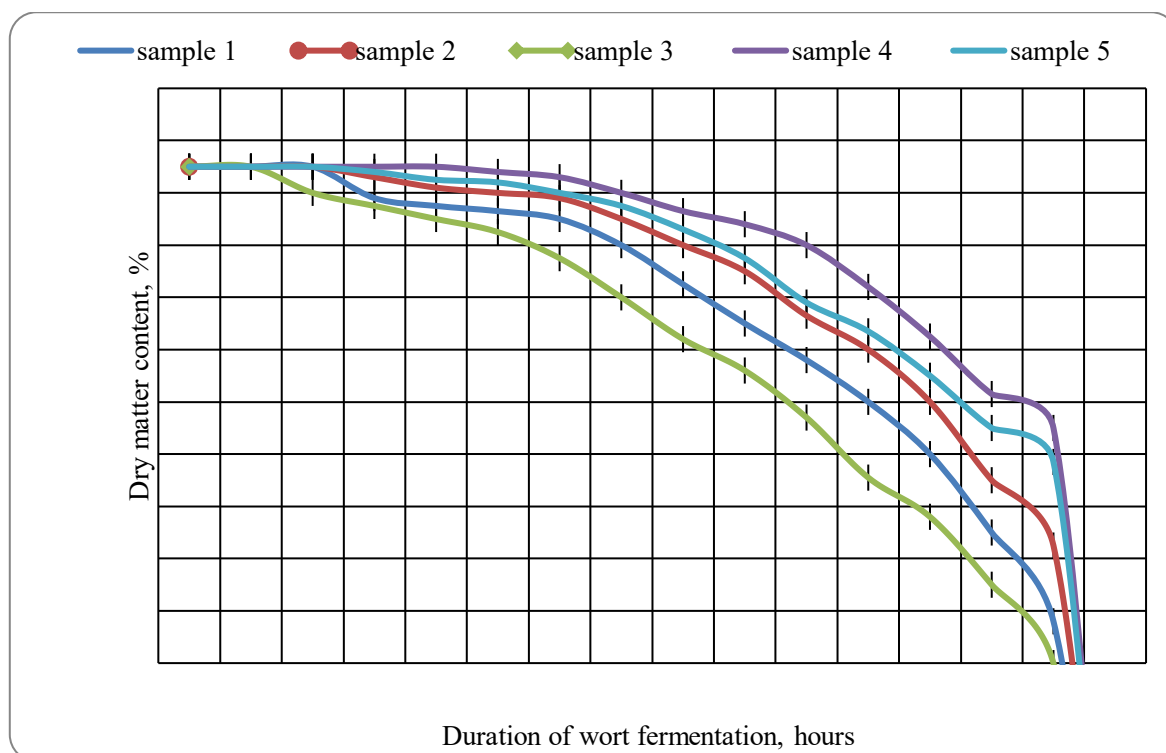


Fig. 1. Dynamics of wort dry matter at a fermentation temperature of 30 °C

It was established that in the first 2.0...2.5 hours of fermentation, the reduction of dry matter content practically did not occur in samples 1, 2. For samples 4 and 5, this duration was about 3...4

hours. This indicates the necessary duration of yeast adaptation to environmental conditions. During the fermentation of sample 3, the lag phase was of insignificant duration. Presumably, this is explained by their greater adaptability to the environment, since the drug is intended for the preparation of bread kvass. Fermentation of the wort in this sample lasted 16 hours. The duration of fermentation of samples 1, 2, 5 and 4 was 17.5; 19; 20 and 22 hours, respectively.

Thus, it was determined that sample 3 can be considered the most acceptable according to the given indicators.

The dynamics of the titrated acidity of the wort at a temperature of 30 °C is shown in Fig. 2.

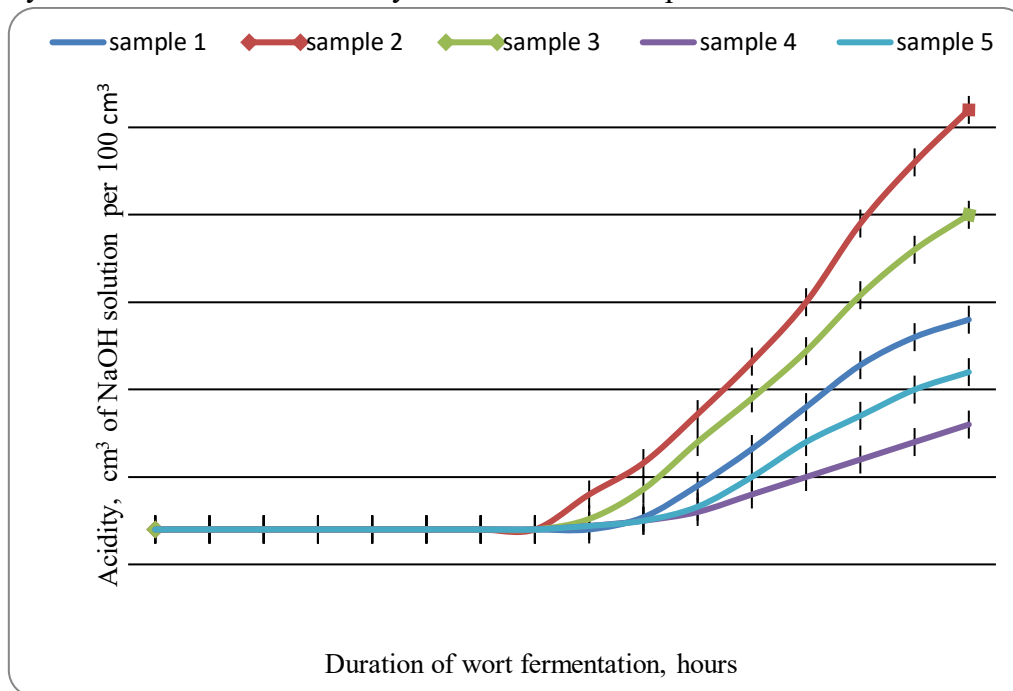


Fig. 2. Dynamics of the titrated acidity of the wort at a fermentation temperature of 30 °C

A low rate of acid formation at 30 °C was noted for all samples. However, in samples 1 and 3, the acidity increased more intensively during 21 and 17 hours, respectively. This can be explained by the presence in their composition of strains of lactic acid bacteria that are able to ferment carbohydrates at low temperatures. The lag in the increase in acidity of sample 4 is explained by the absence of glucose-fermenting bacteria in the sourdough.

Therefore, the moderate fermentation of fermented wort at 30 °C occurs most intensively in samples 1, 2, 3 during 17...21 hours. At this time, there is a decrease in the initial concentration of dry substances by 0.8...1.0% and an increase in acidity to 2 cm³ of NaOH solution per 100 cm³ of kvass.

It can be assumed that for sufficient acid formation in a shorter time, a temperature higher than 30 °C is favorable. Therefore, the next fermentation of the wort was carried out at a temperature of 36 °C.

The change in the content of dry substances and titrated acidity at the fermentation temperature of the wort of 36 °C is shown in Fig. 3 and 4.

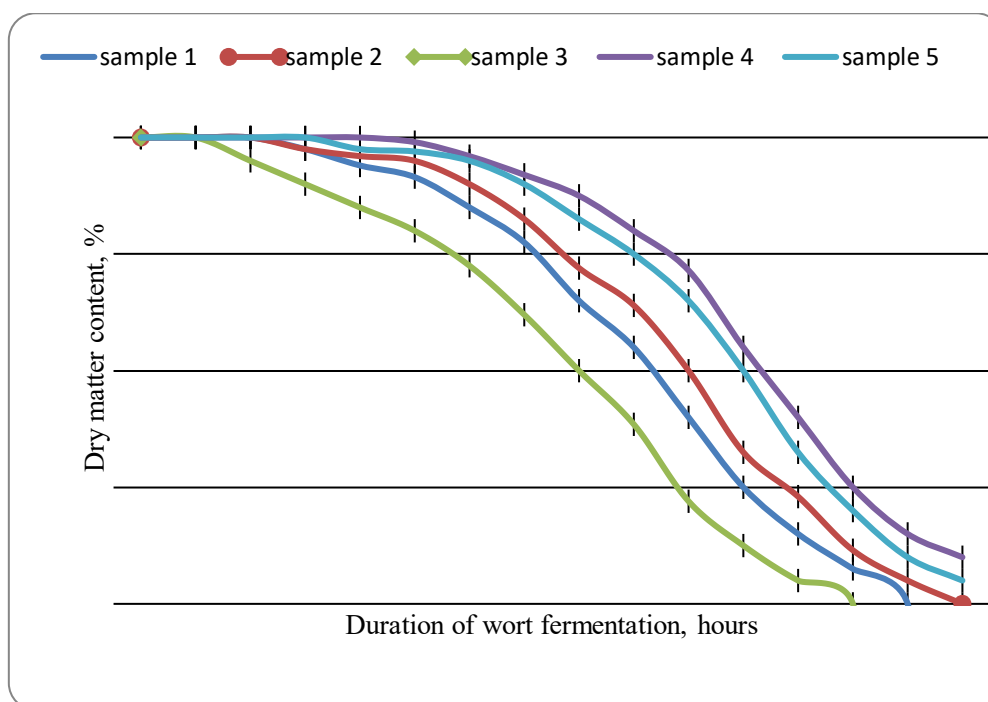


Fig. 3. Dynamics of changes in dry substances at a temperature of 36 °C

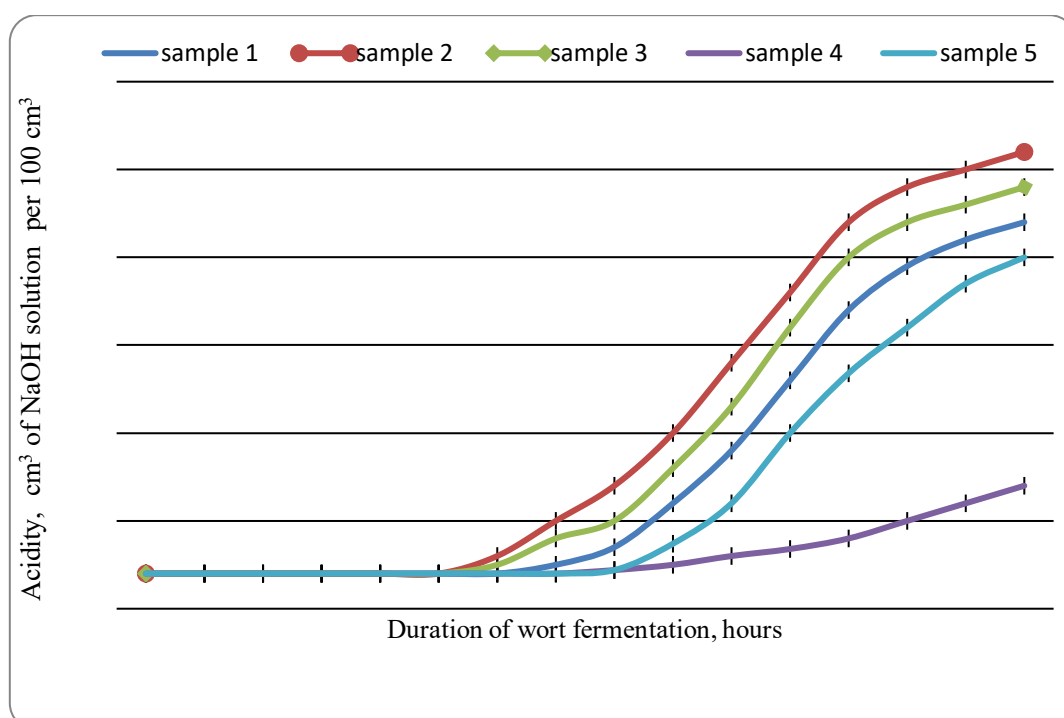


Fig. 4. Dynamics of changes in titrated acidity at a temperature of 36 °C

It was established that at a fermentation temperature of 36 °C, the fermented wort reached the normative indicators of a decrease in the content of dry substances in a shorter period of time than at a fermentation at a temperature of 30 °C. The most intensive fermentation took place in samples 3 (14 h), 1 (16 h) and 2 (18 h), which is explained by the presence in them of the thermotolerant yeast culture *Saccharomyces cerevisiae* MP-10.

Sample 4 (1.7 cm³ of NaOH solution with a concentration of 1 mol/dm³ per 100 cm³) had the

lowest value of the acidity indicator at 36 °C, which is explained by the absence of sugar-fermenting lactic acid bacteria in its composition. Samples 2 (14.5 h), 3 (15 h) and 1 (16 h) best provided the necessary acidity.

Thus, the phase of yeast adaptation to the environment at a higher temperature was short-lived, the fermentation process was shortened by almost 3 hours. The increase in titrated acidity was also more intense when the fermentation temperature increased.

The obtained research results were compared. It was established that the yeast *Saccharomyces cerevisiae* MP-10 is capable of fermenting sourdough wort in the presence of cultures of lactic acid bacteria that are not typical for brewing kvass. Fermentation takes place both at the usual temperature for alcoholic fermentation of 30 °C and at a higher temperature of 36 °C. It should be noted that for most samples, raising the temperature to 36 °C allowed to shorten the fermentation process by 3...5 hours. in comparison with fermentation of wort at a temperature of 30 °C.

The following series of studies was conducted to study the influence of the fermentation temperature regime of sour wort on the accumulation of yeast cells during combined alcoholic and lactic acid fermentation.

It is known that the duration of fermentation is influenced by the concentration of seed microorganisms, their physiological state and fermentation temperature.

In order to find out the influence of lactic acid bacteria on the viability of yeast cells, their physiological state was studied with the determination of the number of dead cells.

Sterile kvass wort with a concentration of 3.3% of dry substances was fermented at temperatures of 30 and 36 °C for 18...24 hours. The initial concentration of yeast in the wort was 4.7 million cells per cm³ of wort.

The content of yeast cells in fermented wort within 18...24 hours. at temperatures of 30 and 36 °C are shown in fig. 5...7.

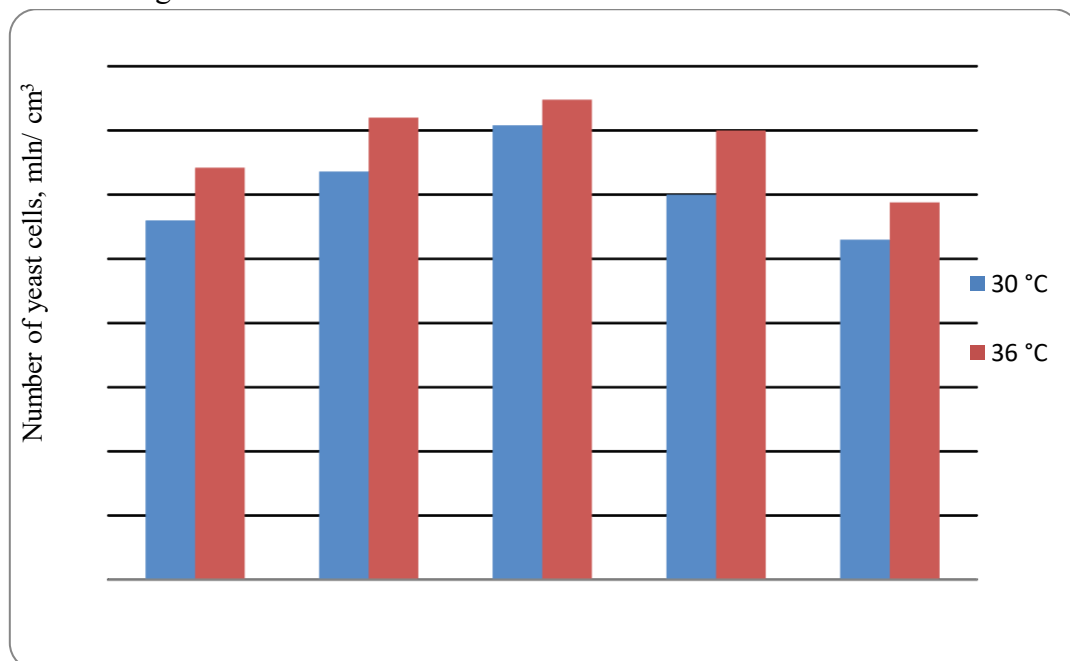


Fig. 5. The effect of temperature on the growth of yeast cells for a fermentation duration of 18 hours.

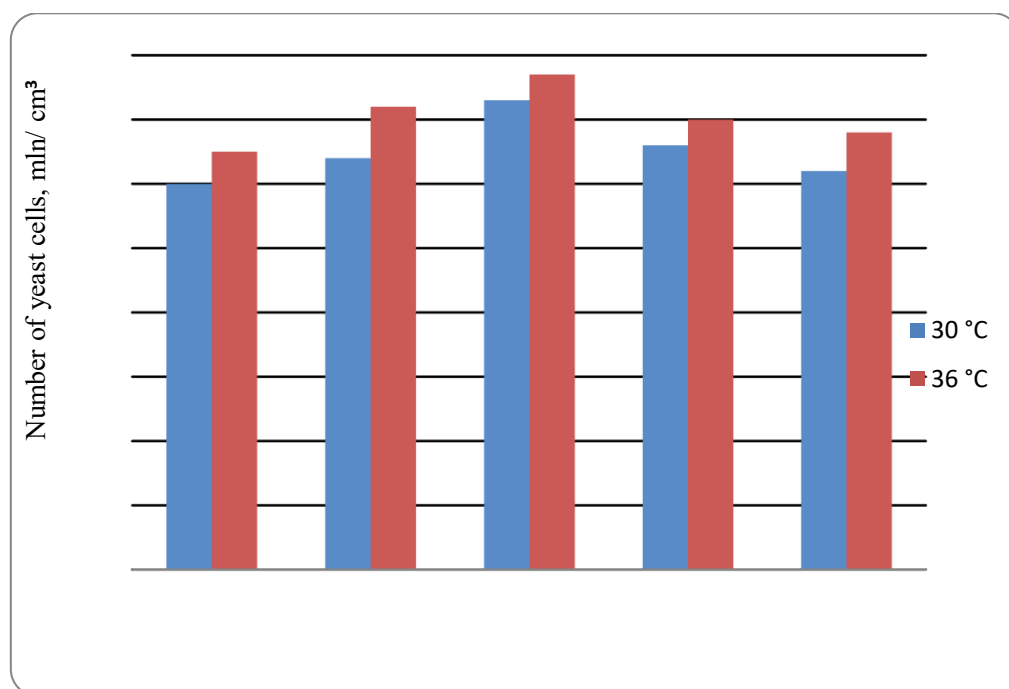


Fig. 6. The effect of temperature on the growth of yeast cells for a fermentation duration of 20 hours.

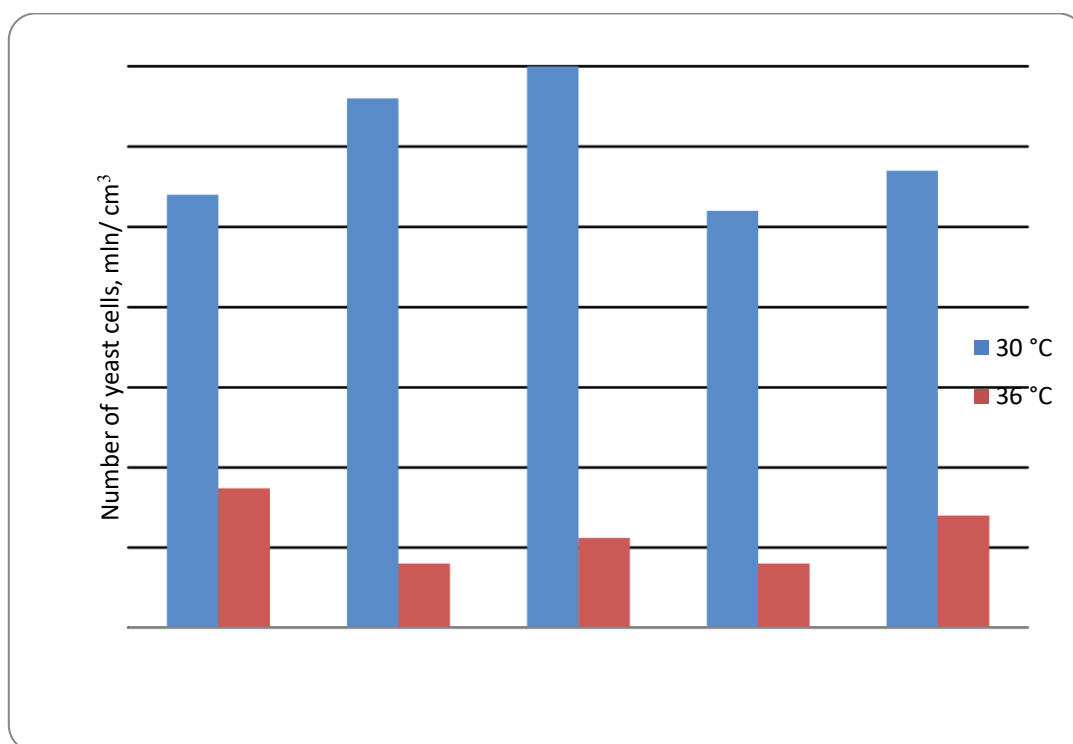


Fig. 7. The effect of temperature on the growth of yeast cells for a fermentation duration of 24 hours.

It was established that the yeast *Saccharomyces cerevisiae* MP-10 in all studied samples had high fermentation activity in the environment with lactic acid bacteria. When the duration of fermentation of wort is 18 hours at different temperatures, an increase in the number of yeast cells

was observed throughout the process. The most intensive accumulation of yeast cells (exponential growth phase) was observed at a temperature of 30 °C. At the same time, up to 40 million cells per cm³ accumulated in the environment. At the same time, the number of dead cells was less than 2%.

An increase in temperature to 36 °C (Fig. 7) led to a shortening of the exponential phase. After the stationary phase, cell growth decreased by 10...12% after 20 hours of fermentation. At this temperature, thermolabile lactobacilli are activated, the environment is depleted, and acidity increases. Therefore, an increase in the number of dead yeast cells up to 20% was observed, which subsequently led to a deterioration of the organoleptic indicators of the finished product due to yeast autolysis.

Therefore, for the fermentation of kvass wort with researched starters, including the use of yeast *Saccharomyces cerevisiae* MP-10, it is possible to recommend a temperature within the range of 30...36 °C. This contributes to the active process of alcoholic fermentation, growth and reproduction of yeast up to 20...25%, improvement of the organoleptic properties of kvass. However, the temperature of 36 °C is the limit and the duration of fermentation should not be more than 18 hours. Based on the obtained results, it was determined that dry preparations of lactic acid bacteria cultures can be used in kvass technology, since the nature of wort fermentation and the increase in acidity did not have significant differences.

The organoleptic characteristics of kvass of the studied samples are given in table. 2.

Table 2

Organoleptic characteristics of kvass for the studied samples

Indicator	Sample №				
	1	2	3	4	5
Appearance	Dark brown color, without turbidity (7.0)	Dark brown color, without turbidity (6.0)	Dark brown color, without turbidity (6.6)	Brown color, cloudy (5.7)	Dark brown color, no cloudiness (4.9)
Flavor	Strong aroma of rye bread, clean, characteristic of bread kvass (5.0)	Pure aroma of rye bread, characteristic of bread kvass (5.0)	the aroma is characteristic of bread kvass, clean (5,0)	aroma of rye bread, characteristic of bread kvass (4.8)	the aroma is uncharacteristic, the extraneous smell of burnt rye bread (3.7)
Taste	Harmonious, strong taste of rye bread, sweet and sour, Refreshing (6.9)	Refreshing, harmonious taste, without extraneous aftertaste (6.0)	The characteristic taste of kvass is strongly expressed, sweet and sour (6.5)	Refreshing taste, without extraneous aftertaste (5,5)	Sour, empty taste, bitterness is felt, not characteristic of kvass (3.6)
General assessment, points	18.9	17.0	18.1	16.0	12.2

According to the results of the organoleptic evaluation of the studied samples, it was established that the appearance and color of the drinks were traditional. They had a characteristic dark brown color, were opaque, and did not contain foreign inclusions. It should be noted that sample 4 had a less saturated color, the aroma of burnt bread and bitterness in the aftertaste. The aroma of some samples was insufficiently pronounced or empty. Most of the samples had a mild, harmonious sweet-sour taste characteristic of bread kvass.

Kvass samples 1 and 3 received the highest score, which indicates the expediency of their use in kvass technology.

Conclusions: 1. The possibility of using dry sourdoughs Acidolact VIVO, Yogurt VIVO, Kvass VIVO, Bifivit VIVO, Streptosan VIVO in the technology of bread sourdough is substantiated.

2. The influence of temperature on the physiological activity of *Saccharomyces cerevisiae* MP-10 yeast during the combined alcoholic and lactic acid fermentation of fermented wort using the studied dry starters was determined.

3. Shortening the fermentation process and obtaining high quality indicators of the finished product is ensured by increasing the fermentation temperature to 36 °C and using the culture of *Saccharomyces cerevisiae* MP-10.

3. The organoleptic indicators of kvass obtained using dry leavens in combination with the use of pure yeast culture *Saccharomyces cerevisiae* MP-10 indicate the feasibility of its use in bread kvass technology.

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